INDOLEACETIC-NICOTINIC ACID INTERACTIONS IN THE ETIOLATED PEA PLANT

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(WITH THREE FIGURES)

Received May 2, 1949

The present author has previously reported that indoleacetic acid (IAA) and nicotinic acid (NA) may interact synergistically in the growth of pure cultures of excised asparagus stem tips (**4**) and under certain conditions in cultures of *Lactobacillus arabinosus* 17–5 (**7**). The present investigations describe further instances of interaction between these compounds, and provide additional data which may contribute to an understanding of the mechanism of the effect.

Because of previous experience with growth responses of etiolated peas (**5, 6**) and because of the known requirement of pea roots for nicotinic acid (**3**), it was considered desirable to utilize etiolated pea plants for the present investigation.

Materials and methods

Alaska peas (*Pisum sativum* L.) were soaked in tap water for two hours and were then sown in flats containing washed river sand. They were allowed to germinate and grow in a dark room whose temperature was 25° C. The only light to which they were exposed prior to harvest was phototropically inactive light transmitted through an orange-red Corning filter (#348).

At the age of seven to eight days after planting, the etiolated pea epicotyls were about 20 cm. tall, and had three nodes on the erect portion of the stem. The two lower nodes produce scales which do not normally expand into leaves. In the axils of these scales are found one or more buds which customarily remain dormant. For the experiments on bud growth herein described, the experimental material consisted of 10 cm. long sections of the epicotyl, containing scale node #2 in the median third of the section. Such epicotyl sections were placed erect in tap water for two hours immediately after cutting to deplete them of reserve materials. They were then placed in groups of 12 into 50 ml. Erlenmeyer flasks containing 5 or 10 ml. of the solution to be tested. The orifice of the flask was closed by the upper end of the epicotyl sections and by a small cotton plug. To avoid undue microbial contamination solutions were replaced every two to three days.

Flasks containing experimental epicotyl sections were either allowed to remain in the dark room or were removed to a light chamber kept at 26° C.

Light of about 500 foot candles intensity was supplied by batteries of 40 Watt "Daylight" and "White" Mazda fluorescent tubes mounted about 12 inches above the experimental shelf.

Experiments on root initiation were conducted by inverting groups of similar epicotyl sections into auxin-containing test solutions for 24 hours in the dark, and then reinverting them into auxin-free solutions containing 2% sucrose. After seven days, numerous roots were apparent at the bases of such epicotyls. These were counted to give a measure of the comparative root-initiating effect of the test solutions.

In order to avoid complications due to differential entry of NA at different pH values, nicotinamide (NAm) was used in place of the free acid. The l(−)tryptophane and nicotinamide used were Merck products; the indoleacetic acid was the Eastman Kodak product, and the l-kynurenine sulphate and 3-hydroxyanthranilic acid were obtained from Drs. H. K. Mitchell and J. F. Nyc of these laboratories.

Assays for nicotinic acid were made by the SNELL and WRIGHT technique (**13**) utilizing *Lactobacillus arabinosus* 17–5 as an assay organism. Auxin assays were conducted by the conventional Avena technique of WENT and THIMANN (**18**).

IAA-NAm interactions in root initiation

WENT (**17**) has shown that IAA and other auxins can cause the initiation of numerous root primordia at the base of pea epicotyl sections. In such tests, it is general to apply the auxin apically, because of the basipetal polarity of transport of this compound. In the experiments outlined below, IAA and NAm were incorporated into the test solutions into which the epicotyls were inverted for 24 hours in the dark. The epicotyls were then removed from the solutions, rinsed free of auxin, and reinverted into auxin-free media containing the experimental concentration of NAm, 2% sucrose and M/15 pH 6.1 phosphate buffer. Duplicate flasks were prepared for this secondary treatment, one set being stored in the dark room, and the other being exposed to the light. The solution was replaced as needed, and the roots counted after the epicotyls had remained in the solution for an additional six days.

As seen from the data of table I, root initiation proceeds well in the dark, but is strongly inhibited by light. Traces of NAm cause the appearance of some roots; these are presumably the pre-existing root primordia of the epicotyls. The addition of auxin results in the formation of new root primordia, and if optimal NAm and IAA concentrations are combined, both the number and length of roots produced are sharply increased, the effect being much greater than a simple additive one. The maximum stimulatory effect of NAm is exerted at about 0.5 γ/ml.: concentrations in the neighborhood of 50 γ/ml. are distinctly inhibitory.

TABLE I

INTERACTIONS OF IAA AND NAm IN THE ROOTING OF ETIOLATED PEA EPICOTYLS. GROUPS OF 10 CM. LONG EPICOTYLS INVERTED INTO 10 ML. OF TEST SOLUTION CONTAINED IN 50 ML. ERLENMEYER FLASKS KEPT IN THE DARK. AFTER 24 HOURS, EPICOTYLS REINVERTED INTO IAA-FREE SOLUTIONS CONTAINING THE SAME NAm CONCENTRATION, 2% SUCROSE AND M/15 pH 6.1 PHOSPHATE BUFFER. ROOTS COUNTED AFTER END OF 7TH DAY OF TREATMENT. DARK = OCCASIONAL WEAK RED LIGHT OF THE AVENA ROOM; LIGHT = 500 F.C. MIXED "WHITE AND "DAYLIGHT" FLUORESCENT LIGHT.

TEST SOLUTION		NUMBER AND LENGTH OF ROOTS PRODUCED			
		DARK		LIGHT	
γ/ML. IAA	γ/ML. NAm	NO. OF ROOTS PER 10 EPICOTYLS	AV. LENGTH (MM.)	NO. OF ROOTS PER 10 EPICOTYLS	AV. LENGTH (MM.)
0	0	0	.....	0	.....
0	0.5	15	4.9	0	.....
0	5.0	0	.....	1	0.2
0	50	2	1.6	0	.....
10	0	18	11.0	8	6.4
10	0.5	53	31.8	0	.....
10	5.0	22	10.2	0	.....
10	50	18	9.1	0	.....

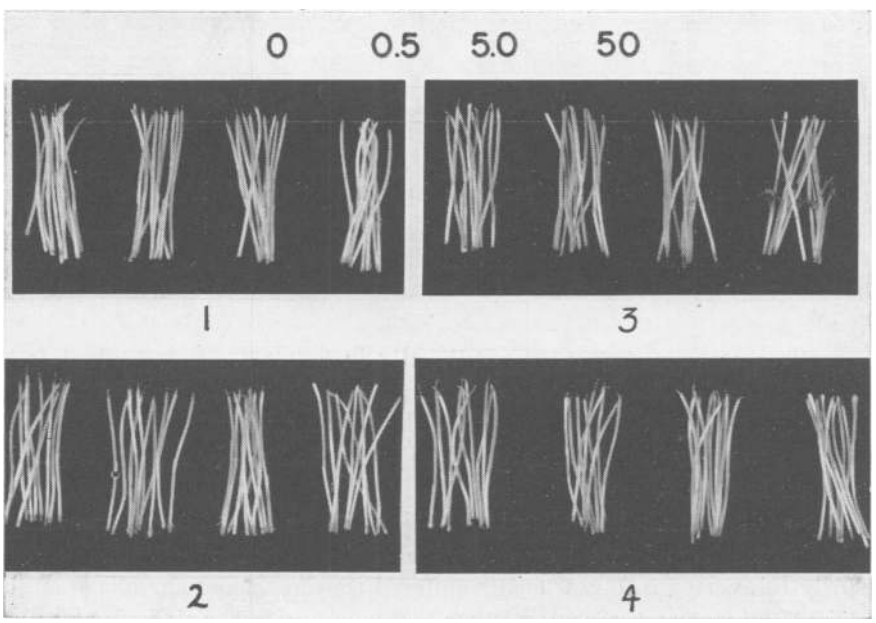

FIG. 1. Interaction of IAA and NAm in bud growth, rooting and apical shrinkage of etiolated pea epicotyls. Description in the text. In each series of four groups of epicotyls the concentrations of NAm were, from left to right, 0, 0.5, 5.0 and 50 γ/ml. 1. Dark O IAA. 2. Dark 10 γ/cc. IAA. 3. Light O IAA. 4. Light 10 γ/cc. IAA.

IAA-NAm interactions in bud growth

It was fortuitously noted that in rooting experiments performed in the light, those test solutions devoid of IAA but containing 50 γ/ml. NAm produced vigorous growth of the normally dormant axillary buds (cf. figure 1). This effect was not apparent if (a) auxin was supplied in the test solution or (b) light was omitted. These results imply that the normal IAA induced-lateral bud inhibition described by Thimann and Skoog (15) is operative, and also that other essential growth factors are synthesized only in the light. Data obtained by weighing or measuring the length of the buds produced are shown in table II.

TABLE II

IAA–NAm interactions in bud growth of etiolated peas. Procedural details as in table I. All experiments conducted in the light

Test solution		Fresh wt. (mg.) of buds per 10 epicotyls						Av. length of buds
		Expt. P-33*		Expt. P-37		Expt. P-38		Expt. P-67
γ/ml. IAA	γ/ml. NAm	Dark	Light	Dark	Light	Dark	Light	Light
0	0	25	25	21	29	16	58	3.1 ± 0.45
0	0.5	15	30	.....	.....	.....	.....	
0	5.0	10	25	.....	.....	.....	.....	
0	50	30	*180*	17	*78*	16	*119*	7.0 ± 1.4
10	0	15	15	.....	.....	11	18	
10	0.5	10	15	.....	.....	.....	.....	
10	5.0	15	15	.....	.....	.....	.....	
10	50	15	15	14	19	13	13	

* Weight to nearest 5 mg.

Since NAm obviously stimulates bud growth and IAA inhibits bud growth it was thought desirable to set up an experiment in which IAA and NAm levels were simultaneously varied over large ranges. In such a way a more quantitative picture of the nature of the interaction could be obtained.

Typical data are presented graphically in figure 2. It is evident from this experiment that at any IAA level, bud growth is most stimulated by 25 γ/ml. NAm, but the range over which NAm may exert its effect is more or less limited by the IAA level. The implications of such data will be discussed later in the paper.

One additional finding of unknown significance should be reported here. As is obvious from figure 1 epicotyls inverted into test solutions and subsequently reinverted into new media suffered a necrosis and shrinkage of the previously immersed apical portion. However, if 50 γ/ml. NAm were present in the test solution bathing the immersed area, no shrinkage resulted, and in some instances, an actual enlargement was produced. This

effect of NAm, unlike the bud growth effect, is independent of IAA and of illumination.

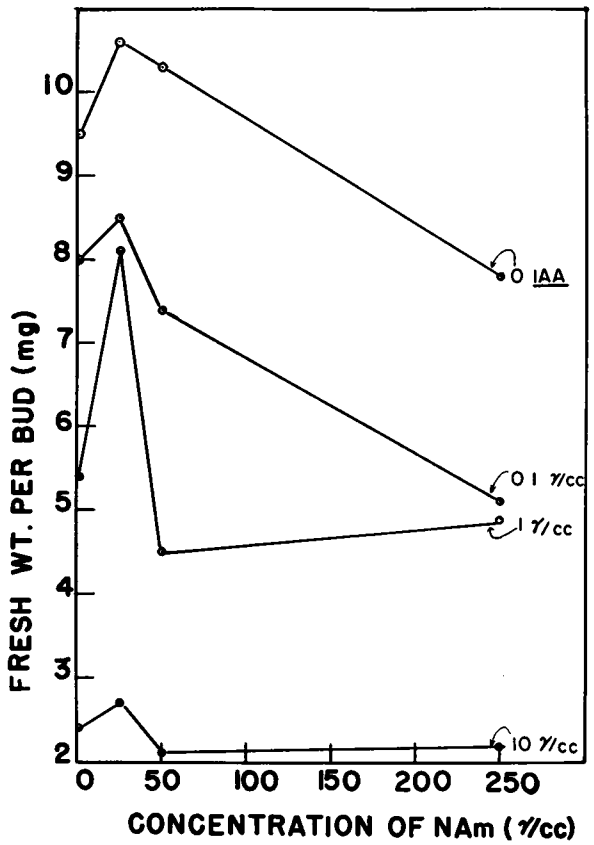

FIG. 2. Quantitative interaction between NAm and IAA in the bud growth test.

MECHANISM OF THE INTERACTION

When one attempts to explain this type of interaction between IAA and NA, one may make a working hypothesis on the basis of recent biochemical findings. For instance both IAA (**16**) and NA (**9, 10**) are known to arise from tryptophan in various organisms. The transformations leading to IAA production from tryptophan apparently proceed *via* tryptamine or indolepyruvic acid (**8**), whereas those leading to NA production from tryptophan proceed *via* kynurenine and 3-hydroxyanthranilic acid (**2, 11**). Since both IAA and NA may arise from tryptophan, and since both are physiologically active, the synergistic effect could be explained in terms of a sparing action. Thus, the addition of exogenous NA would make possible a greater diversion of tryptophan to IAA, resulting in an apparently enhanced IAA effect. To test the applicability of this hypothesis to the

etiolated pea plant, it was necessary to conduct experiments to determine whether the hypothecated conversions actually do occur.

The conversion of tryptophan to auxin

Epicotyls were harvested and cut into pieces about 1 cm. long. Aliquots of the pooled tissue were then removed and incubated in control solutions (2% sucrose and M/15 pH 6.1 phosphate buffer) or in experimental solutions (the same solution fortified with 250 mg/L 1(−)tryptophan). After 16 hours, the tissue was gathered, rinsed 3 times with redistilled water, frozen in liquid air, and dried in a lyophil apparatus. The dried tissues were ground to 40 mesh in a small Wiley mill, and 50 mg. samples extracted for two hours with ice-cold peroxide-free ethyl ether. The ether extracts were then assayed for auxin by the conventional Avena coleoptile curvature test (**18**). Typical results are shown in table III. It is quite clear that

TABLE III

The conversion of 1(−)tryptophan to auxin by etiolated pea tissue. Description in the text

Treatment	Degrees curvature ± standard error		
	Row A	Row B	Average
Control	2.2 ± 0.5	2.1 ± 0.3	2.2
+ 250 mg/L 1(−)tryptophan	11.9 ± 1.0	19.4 ± 1.0	15.7

the addition of tryptophan to the medium results in higher ether-extractable auxin in the tissues. This is presumably due to the formation of IAA.

The conversion of precursors to nicotinic acid

Epicotyl tissue was cut into 1 cm. lengths and 500 mg. fresh wt. immersed in 10 ml. of solution containing a presumed precursor. The following concentrations were used: 1(−)tryptophan 250 mg/L; 1-kynurenine sulphate 100 mg/L; 3-hydroxyanthranilic acid 50 mg/L. After 24 hours, the tissue was removed, rinsed, covered with 1 N H_2SO_4 and hydrolyzed in an autoclave for 30 min. at 15# pressure. After cooling, the hydrolyzate was filtered free of tissue fragments, adjusted to pH 6.8 and made up to standard volume. Aliquots were then assayed for nicotinic acid.

In the assay, 5 ml. of NA-free medium was added to 5 ml. of solution to be assayed, then inoculated with one drop of a 24-hour old culture of *L. arabinosus* 17–5. The tubes were incubated at 30° C for 72 hours, and the lactic acid titrated with 0.1 N NaOH, using bromthymol blue as an indicator. A standard series was run along with the unknowns, and nicotinic acid in each tube determined by reference to the standard curve. In general, the utilizable assay range was .05 – 0.4 γ nicotinamide per tube. Despite repeated trials, evidence could be found only for a slight conver-

sion of tryptophan to NA. However, kynurenine and especially hydroxyanthranilic acid seemed definitely to be readily converted to a material active as NA in the bioassay. Results are summarized in table IV.

TABLE IV

THE EFFECT OF VARIOUS PRESUMED PRECURSORS ON THE NA CONTENT OF PEA EPICOTYL TISSUE. DESCRIPTION IN THE TEXT

EXPERIMENT #	γ NA PER GM. DRY WEIGHT OF TISSUE				
	CONTROL	250 MG/L 1(−)TRYPTOPHAN	100 MG/L 1-KYNURENINE SULPHATE	50 MG/L 3-HYDROXY-ANTHRANILIC ACID	50 MG/L NA
P-256	45	55	71	225	259
P-243	84	89	100	194	207
P-158	33	33	.....	.....	400
P-152	33	50	.....	.....	150

PHYSIOLOGICAL EFFECTS OF IAA- AND NA-PRECURSORS

If tryptophan may be converted to IAA and to NA as indicated by the previous experiments, then it should also exert physiological effects produced by these end products. Similarly, kynurenine and hydroxyanthranilic acid should produce the same general effects as does NA. That these effects are in fact produced is evident from the following experiments.

Precursors were incorporated into test solutions in the concentrations

TABLE V

THE EFFECT OF PRESUMED PRECURSORS OF NA ON THE GROWTH OF ETIOLATED PEA EPICOTYL BUDS IN THE LIGHT. EPICOTYLS EITHER ERECT OR INVERTED DURING THE FIRST 24 HOURS; ALL ERECT DURING FINAL SIX DAYS; HARVEST AT END OF SEVENTH DAY

TEST SOLUTION	EPICOTYLS ERECT OR INVERTED DURING 1ST 24 HOURS	FRESH WEIGHT OF BUDS PER 10 EPICOTYLS (MG.)
Water	Inverted	67.3
500 γ/ml. 1(−)tryptophan	"	9.4
200 γ/ml. 1-kynurenine sulphate	"	92.0
100 γ/ml. 3-hydroxy-anthranilic acid	"	108.0
50 γ/ml. NAm	"	123.2
Water	Erect	19
500 γ/ml. 1 (−)tryptophan	"	81
50 γ/ml. NAm	"	84

indicated. The usual bud growth test was performed, all flasks being exposed continuously to 500 fc of light. After seven days, the buds were excised and weighed. Data are presented in table V.

It is obvious that kynurenine, hydroxyanthranilic acid and nicotinamide, when applied apically, all stimulated bud growth. Tryptophan when applied apically was inhibitory, but when applied basally was stimulatory; indicating that it may produce both IAA and NA. Under conditions where IAA may migrate to the bud (basipetally) the net effect is an inhibition: under conditions where IAA cannot migrate to the bud (acropetally) the net effect is stimulation. In subsequent experiments, it was also shown that tryptophan may result in enhanced root initiation, further indicating that the tryptophan ⟶ IAA transformation does occur.

Discussion

On the basis of the data presented it seems valid to conclude that (a) various physiological interactions between IAA and NA may be demonstrated in the etiolated pea plant and (b) these interactions are probably due to a sparing action, since both NA and IAA are produced from a common precursor, tryptophan (figure 3). Thus, the addition of either one

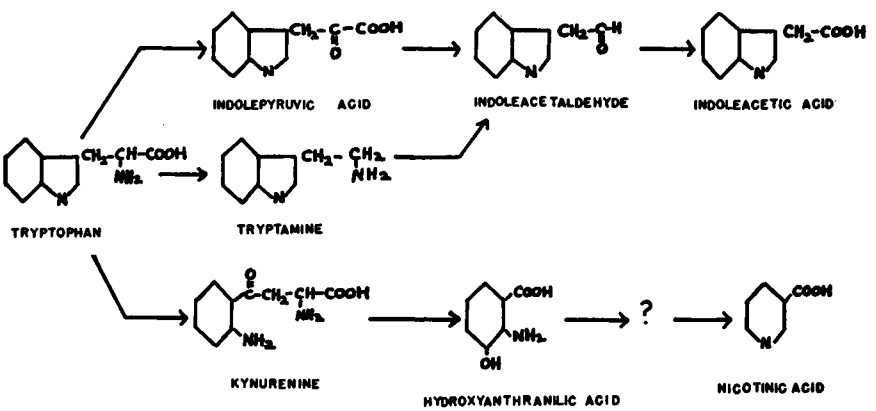

FIG. 3. Summary of pathways of conversion of tryptophan to nicotinic acid and to indoleacetic acid.

makes available more tryptophan for conversion to the other, effectively increasing the concentration of both constituents by the addition of one. Whether synergism or antagonism is to result from such an interaction will depend on the ratio of these two products formed and upon the organ involved.

AUDUS (1) reports an inability to detect IAA-NA interactions in seedlings of water cress. Since the tested seedlings were evidently connected with their cotyledons, it seems that failure to obtain positive results stemmed from a failure to deplete endogenous reserves of both NA and IAA.

Under such conditions, addition of small quantities of exogenous NA would not be expected to yield discernible results.

It should also be noted that although TERROINE (**14**) reports an inability of cultured *Phaseolus multiflorus* embryos to form NA from tryptophan, NASON (**12**) reports success in a similar experiment with *Zea mays*. In neither case did the experimenter attempt to utilize any intermediates between tryptophan and NA.

It may be significant for the problem of lateral bud inhibition that a single substrate (tryptophan) may be metabolized to two different end products, one of which (NA) may stimulate bud growth and the other of which (IAA) inhibits bud growth. It would appear advisable to conduct similar investigations on other plants, to see whether the inhibition imposed by IAA may be reversed by proper levels of NA or other bud growth factors. In this connection, it is necessary to recall that previous investigations (**6**) have demonstrated that adenine may also serve as a bud growth factor in etiolated peas. NA may apparently substitute for adenine; the maximum rate of bud growth produced by either of these materials is not further increased by the addition of the other. The mechanism of this effect is as yet not clear; but it is apparent that the demonstration that presumed NA precursors may stimulate bud growth is not definitive proof of their conversion to NA. However, when such evidence is supported by evidence from microbioassays, there can be little doubt that the transformations actually do occur.

Summary

Various physiological interactions between indoleacetic acid (IAA) and nicotinic acid (NA) have been demonstrated in the etiolated pea epicotyl. For example, NA enhances the root initiation activity of IAA (synergism), but IAA reverses the bud-growth stimulation produced by NA (antagonism). Evidence from bioassays indicates that tryptophan may be converted rapidly to IAA and slowly to NA, the latter conversion apparently occurring through kynurenine and 3-hydroxyanthranilic acid as intermediates. It is suggested that the various IAA-NA interactions may be explained on the basis of their origin from a common precursor, tryptophan.

The author wishes to express appreciation to Miss Margery Hand for aid rendered during the course of this investigation.

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